Biochemistry

INTERACTIONS OF gp91^{phox} AND p47^{phox} OF THE NADPH OXIDASE

Rebecca M. Kerkhoff, Colleen B. Tomcik[1], Brendan A. LeRoy[1], Karla J. Biberstine-Kincade[1], Mary C. Dinauer[1], Mary K. Ritke[2]*
1 Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, IUPUI, Indianapolis, Indiana 46202; wellsadm@iupui.edu
2 University of Indianapolis, 1400 E. Hanna Ave., Indianapolis, Indiana 46227; mritke@uindy.edu

The NADPH oxidase is composed of four protein subunits: gp91^{phox}, p47^{phox}, p67^{phox}, and p22^{phox}, where *phox* stands for *ph*agocyte *ox*idase. When stimulated, these subunits form the functional oxidase and produce superoxide (O_2^-), a precursor of microbicidal oxidants. We transiently transfected p47^{phox} wild type and seven mutants individually into two types of cos cells--cos 3, which contain wild type gp91^{phox}, p67^{phox}, and p22^{phox}, and cos E, which contain gp91^{phox} R91/92E and wild type p67^{phox} and p22^{phox}. The mutants consisted of changing three or six acidic amino acids in an acidic region to basic or neutral or charge flipping various amino acids in three Pick regions (regions identified by Edgar Pick to be important in the interaction of p47^{phox} with gp91^{phox}). Our hypothesis was that the mutants with charge flipped amino acids would interrupt oxidase function in cos 3 cells, but would rescue it in cos E cells. Following the transfection, we harvested the cells and performed several assays. Western blots were run to check the expression of each protein. NBT tests and cytochrome c assays were performed to test for superoxide production, the sign of a functional oxidase. We found that all proteins, wild type and mutant, were expressed. NBT tests were positive for cos 3 cells with wild type p47^{phox} and all mutants except for those which changed six amino acids in the acidic region. Those that changed only three amino acids in the acidic region had approximately half the activity of the wild type. NBT tests were negative for all cos E cells, with the possible exception of one mutant where six amino acids in the acidic region were charge flipped. The results of the cytochrome c assays were below the level of detection.